

### REMARKS

Claims 36-41 were examined in the Office Action mailed September 26, 2002. As indicated above, claims 39-41 have been cancelled from the application. These claims are cancelled without prejudice and applicant reserves the right to pursue these claims in a separately filed application. In addition, new claims 42-49 have been added to the application as indicated above.

As requested by the examiner, the specification has been amended to update the statement of priority.

Figure 2 has been cancelled from the application. The specification has been amended accordingly as indicated above to delete descriptions and all references to this figure. In addition, Figure 3 has been renumbered as "Figure 2" and the specification amended accordingly as indicated above. A substitute version of this latter figure with a corrected caption is submitted herewith and entry of this corrected figure into the application is requested. The results that were illustrated in cancelled Figure 2 are described in Example 13 of the specification. The cancellation of this figure is believed not to impact the enablement or description of the claimed invention, and does not introduce new matter into the application.

The examiner has objected to the disclosure because the specification in several instances refers to amino acid sequences by referring to SEQ ID NOS:10 and 12, which show both nucleotide and amino acid sequences. The examiner has asked that these references be changed to SEQ ID NOS:11 and 13, which show amino acid sequences only. Although the original references to SEQ ID NOS:10 and 12 do disclose amino acid sequences and are deemed to be correct, these changes have been made to accommodate the examiner's request. These changes do not constitute the addition of new matter to the application.

In addition, a typographical error in the specification at page 36, line 1 has been corrected by amendment as shown above. Here, the text was changed to read "Table 2" instead of "Table 1." It is self-evident from the context that the applicant had intended to refer to Table 2, not Table 1. For example, the text at page 36, line 1 reads "Table 1 below," and the only table located "below" is Table 2. Thus, this amendment does not constitute the addition of new matter to the application.

The examiner has objected to the Abstract of the Disclosure on the basis that it does not pertain to the claimed invention. Accordingly, the Abstract has been changed to reflect the subject matter of the claims currently being sought. The new abstract is supported throughout the specification, for example, at page 4, lines 16-20; page 4, line 36 to page 5, line 2; page 9, lines 26-29; page 11, lines 11-13; page 12, lines 5-7; page 18, lines 2-3; and Example 10 at pages 27-28. Thus, this amendment does not constitute the addition of new matter to the application.

Claim 38(g) has been amended to delete the language referring to hybridization and the deleted limitation replaced with a recitation stating that the RANKL polypeptide is at least 90% identical to the RANKL protein of SEQ ID NO:13. This amendment is supported in the specification, for example, at page 10, line 38 to page 11, line 3. Claim 38 has been further amended to correct a minor grammatical error by inserting the word "and" following part (f) of the claim. These amendments to claim 38 do not constitute the addition of new matter to the application.

New claims 42-49 have been added to the application. Support for new claims 42-47 is found throughout the specification, for example, in pending claim 38; at page 4, lines 16-20; page 4, line 36 to page 5, line 2; page 5, lines 16-19 and 27-33; page 9, lines 26-29; page 11, lines 11-13; page 12, lines 5-7; page 18, lines 2-3; Example 10 at pages 27-28; and page 33, lines 25-29 and 33-36. New claims 48 and 49 are supported, for example, at page 18, lines 2-3 and in Example 10, at pages 27-28. Thus, these new claims do not constitute the addition of new matter to the application:

The examiner has asserted that the oath or declaration is defective because of deficiencies in its recitations of various priority documents. To rectify these deficiencies, a newly executed declaration is submitted herewith.

The examiner has noted that the application was filed with informal drawings. Formal drawings are submitted herewith. She is asked to acknowledge their receipt and to enter these new drawings into the application.

#### Rejections under 35 U.S.C. § 112, First Paragraph

Claims 38 and 41 have been rejected under 35 U.S.C. § 112, first paragraph, on the basis that claims 38(g) and 41(f) are not enabled unless the RANKL polypeptides recited in these claims possess biological activity, such as the ability to bind RANK. Claim 41 has been cancelled from the application, thus this ground for rejection is moot

as regards this claim. The applicant disagrees with this ground for rejection because claim 38 describes methods for raising RANKL-specific antibodies according to claim 36, thus the polypeptides specified in claim 38(g) need only be capable of eliciting such antibodies. However, to accommodate the examiner's concerns about the hybridization language of claim 38(g), this language has been deleted and replaced with language limiting the RANKL polypeptide to one that is at least 90% identical to the human RANKL of SEQ ID NO:13. The examiner has further rejected claim 38 under 35 U.S.C. § 112 on the ground that the hybridization language was not enabled unless further limited to the "complement" of the sequences of SEQ ID NO:12. As the hybridization language has been deleted from the claim, this concern is now moot. As claims 39-41 have been cancelled from the application, this ground for rejection also is moot as applied to these claims. Accordingly, the examiner is respectfully requested to withdraw the rejection of claim 38 based on 35 U.S.C. § 112, first paragraph.

Rejections under 35 U.S.C. § 112, Second Paragraph

Claims 36-41 stand rejected under 35 U.S.C. § 112, second paragraph, in view of the examiner's belief that it is not clear what is meant by the phrase "is immunoreactive with a RANKL polypeptide." As claims 39-41 have been cancelled from the application, this ground for rejection is moot as applied to these claims. The applicant believes that one skilled in the art would readily recognize that an antibody "immunoreactive" with RANKL, as recited in claim 36, is an antibody that can bind RANKL. Nonetheless, to accommodate the examiner's concern, claim 36 has been amended to specify that the claimed antibody "binds" RANKL instead of reciting that the antibody is "immunoreactive with" RANKL. In view of this amendment, the examiner is asked to withdraw the rejection of claim 36 and its dependent claims, claims 37 and 38, under 35 U.S.C. § 112, second paragraph.

**CONCLUSION**

Claims 36-38 and 42-49 are now pending in the application. In view of the amendments and remarks set forth above, these claims are believed to be in condition for acceptance and notification to this effect is respectfully requested.

The examiner is asked to take note that this amendment is accompanied by a submission of formal drawings and a petition to correct inventorship. The examiner is respectfully asked to consider and grant the petition to correct inventorship without delay.

If any issues remain in the application, the examiner is asked to contact the undersigned at her direct dial number given below.

Respectfully submitted,



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#### CERTIFICATE OF MAILING

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Commissioner for Patents, Washington, D.C. 20231, on the date indicated below.

Date: January 24, 2003



D. F. Lindholm

## APPENDIX TO AMENDMENT

(marked-up version of section of specification amended in attached Amendment)

Specification, page 3, lines 4-8:

This application is a divisional of USSN 09/577,780, filed May 24, 2000, now U.S. Patent No. 6,419,929, which is a divisional of USSN 08/995,659, filed December 22, 1997, now U.S. Patent No. 6,242,213, which claims the benefit of USSN 60/064,671, filed October 14, 1997, USSN 60/077,181, filed March 7, 1997 (filed originally as USSN 08/813,509), and USSN 60/059,978, filed December 23, 1996 (filed originally as USSN 08/772,330).

Specification, page 3, line 4:

-- Figure [3] 2 demonstrates that RANKL enhances DC allo-stimulatory capacity. Allogeneic T cells were incubated with varying numbers of irradiated DC cultured as described in Example 13. The cultures were pulsed with [<sup>3</sup>H]-thymidine and the cells harvested onto glass fiber sheets for counting. Values represent the mean  $\pm$  standard deviation (SD) of triplicate cultures. --

Specification, page 5, line 3-34:

-- Soluble forms of RANKL are also within the scope of the invention. The nucleotide and predicted amino acid sequence of the RANKL is shown in SEQ ID [NOs:10 and 12] Nos:11 and 13 (murine and human, respectively). Computer analysis indicated that the RANKL is a Type 2 transmembrane protein; murine RANKL contains a predicted 48 amino acid intracellular domain, 21 amino acid transmembrane domain and 247 amino acid extracellular domain, and human RANKL contains a predicted 47 amino acid intracellular domain, 21 amino acid transmembrane domain and 249 amino acid extracellular domain.

Soluble RANKL comprises a signal peptide and the extracellular domain or a fragment thereof. An exemplary signal peptide is that shown in SEQ ID NO:9; other signal (or leader) peptides are well-known in the art, and include that of murine Interleukin-7 or human growth hormone. RANKL is similar to other members of the TNF family in having a region of amino acids between the transmembrane domain and the receptor binding region that does not appear to be required for biological activity; this is referred to as a 'spacer' region. Amino acid sequence alignment indicates that the

receptor binding region is from about amino acid 162 of human RANKL to about amino acid 317 (corresponding to amino acid 139 through 294 of murine RANKL, SEQ ID [NO:10] NO:11), beginning with an Ala residue that is conserved among many members of the family (amino acid 162 of SEQ ID [NO:12] NO:13).

Moreover, fragments of the extracellular domain will also provide soluble forms of RANKL. Those skilled in the art will recognize that the actual receptor binding region may be different than that predicted by computer analysis. Thus, the N-terminal amino acid of a soluble RANKL is expected to be within about five amino acids on either side of the conserved Ala residue. Alternatively, all or a portion of the spacer region may be included at the N-terminus of a soluble RANKL, as may be all or a portion of the transmembrane and/or intracellular domains, provided that the resulting soluble RANKL is not membrane-associated. Accordingly, a soluble RANKL will have an N-terminal amino acid selected from the group consisting of amino acids 1 through 162 of SEQ ID [NO:12] NO:13 (1 through 139 of SEQ ID [NO:10] NO:11). Preferably, the amino terminal amino acid is between amino acids 69 and 162 of SEQ ID [NO:12] NO:13 (human RANKL; amino acids 48 and 139 of SEQ ID [NO:10] NO:11). Similarly, the carboxy terminal amino acid can be between amino acid 313 and 317 of SEQ ID [NO:12] NO:13 (human RANKL; corresponding to amino acids 290 through 294 of SEQ ID [NO:10] NO:11). Those skilled in the art can prepare these and additional soluble forms through routine experimentation. --

Specification, page 30, line 36:

-- Addition of RANKL to DC cultures significantly increased the degree of DC aggregation and cluster formation above control cultures, similar to the effects seen with CD40L [(Figure 2)]. Sorted human CD1a<sup>+</sup> DC were cultured in a cytokine cocktail (GM-CSF, IL-4, TNF- $\alpha$  and FL) [(upper left panel)], in cocktail plus CD40L (1 $\mu$ g/ml) [(upper right)], in cocktail plus RANKL (1 $\mu$ g/ml) [(lower left)], or in cocktail plus heat inactivated ( $\Delta$ H) RANKL (1 $\mu$ g/ml) [(lower right)] in 24-well flat bottomed culture plates in 1 ml culture media for 48-72 hours and then photographed using an inversion microscope. An increase in DC aggregation and cluster formation above control cultures was not evident when heat inactivated RANKL was used, indicating that this effect was dependent on biologically active protein. However, initial phenotypic analysis of adhesion molecule expression indicated that RANKL-induced clustering was not due to increased levels of CD2, CD11a, CD54 or CD58. --

Specification, page 31, lines 11-24:

-- The addition of RANKL to CD1a<sup>+</sup> DC enhanced their allo-stimulatory capacity in a mixed lymphocyte reaction (MLR) by at least 3- to 10-fold, comparable to CD40L-cultured DC [(Figure 3)] (Figure 2). Allogeneic T cells (1x10<sup>5</sup>) were incubated with varying numbers of irradiated (2000 rad) DC cultured as indicated above [for Figure 2] in 96-well round bottomed culture plates in 0.2 ml culture medium for four days. The cultures were pulsed with 0.5 mCi [<sup>3</sup>H]-thymidine for eight hours and the cells harvested onto glass fiber sheets for counting on a gas phase  $\beta$  counter. The background counts for either T cells or DC cultured alone were <100 cpm. Values represent the mean  $\pm$  SD of triplicate cultures. Heat inactivated RANKL had no effect. DC allo-stimulatory activity was not further enhanced when RANKL and CD40L were used in combination, possibly due to DC functional capacity having reached a maximal level with either cytokine alone. Neither RANKL nor CD40L enhanced the *in vitro* growth of DC over the three day culture period. Unlike CD40L, RANKL did not significantly increase the levels of HLA-DR expression nor the expression of CD80 or CD86. --

Specification, page 35, line 34 to page 36, line 2:

-- Comparison of the nucleotide sequence of murine and human RANK indicated that there were several conserved regions that could be important for TRAF binding. Accordingly, a PCR-based technique was developed to facilitate preparation of various C-terminal truncations that would retain the conserved regions. PCR primers were designed to introduce a stop codon and restriction enzyme site at selected points, yielding the truncations described in [Table 1] Table 2 below. Sequencing confirmed that no undesired mutations had been introduced in the constructs. --

In the Drawings:

In Figure 3, please delete the caption "Figure 3" and substitute therefor -- Figure 2 --.

In the Claims:

36. (once amended) An antibody that [is immunoreactive with] binds a RANKL polypeptide as shown in SEQ ID NO:13.

38. (once amended) A method for preparing an antibody according to claim 36, wherein the antibody is elicited by immunizing with a RANKL polypeptide selected from the group consisting of:

- a) a polypeptide comprising amino acids 1-317 of SEQ ID NO:13;
- b) a polypeptide comprising amino acids 69-313 of SEQ ID NO:13;
- c) a polypeptide comprising amino acids 1-162 of SEQ ID NO:13;
- d) a polypeptide comprising amino acids 162-313 of SEQ ID NO:13;
- e) a polypeptide comprising amino acids 138-317 of SEQ ID NO:13;
- f) a polypeptide comprising amino acids x to y of SEQ ID NO:13, wherein x is an amino terminal amino acid between 69 and 162 of SEQ ID NO:13, and y is a carboxy terminal amino acid between 313 and 317 of SEQ ID NO:13; and
- g) a polypeptide [encoded by a nucleic acid molecule that is capable of hybridizing under stringent conditions to a DNA having a nucleotide sequence as shown in SEQ ID NO:12 or its complement, wherein stringent conditions comprise hybridizing in 6 X SSC at 63°C and washing in 3 X SSC at 55°C] that is at least 90% identical to amino acids 1-317 of SEQ ID NO:13.

In the Abstract:

Provided herein are antibodies that bind human RANKL polypeptides. [Isolated ligands, DNAs encoding such ligands, and pharmaceutical compositions made therefrom, are disclosed. The isolated ligands can be used to regulate an immune response. The ligands are also useful in screening for inhibitors thereof.]